BIOLOGY OF REPRODUCTION **64,** 1614–1618 (2001)

Developmental Changes in the Long Form Leptin Receptor and Related Neuropeptide Gene Expression in the Pig Brain[1]

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ABSTRACT

The hypothalamus is the key site of central regulation of energy homeostasis, appetite, and reproduction. The long form leptin receptor (Ob-Rl) is localized within the hypothalamus along with several neuropeptides that are involved in regulation of the neuroendocrine axis. In the present study, developmental changes in gene expression of the Ob-Rl, preproorexin, proopiomelanocortin (POMC), corticotropin releasing factor (CRF), somatostatin, and GnRH in the hypothalamus was studied. Expression of Ob-Rl and neuropeptide mRNA was examined by semiquantitative reverse transcription-polymerase chain reaction in hypothalami collected from 106-day-old fetus (n = 3) and 7-day-old (n = 3), 3.5-mo-old (n = 3), and 6-mo-old (n = 2) gilts. In addition, leptin mRNA expression in the first three ages was examined in back fat. Leptin mRNA expression increased ($P < 0.05$) by 7 days postnatal, but Ob-Rl mRNA expression increased ($P < 0.01$) by 3.5 mo. Expression of preproorexin ($P < 0.05$), somatostatin, and GnRH ($P < 0.01$) mRNA peaked by 3.5 mo of age while POMC mRNA expression increased markedly ($P < 0.01$) by 6 mo of age. The CRF mRNA expression did not change across ages. These findings suggest a possible relationship among Ob-Rl and a number of hypothalamic and peripheral peptides in the development of the neuroendocrine axis. These peptides may serve as messengers that link mechanisms that regulate reproduction and energy balance.

developmental biology, hypothalamic hormones, hypothalamus, leptin, leptin receptor, neuropeptides

INTRODUCTION

The hypothalamus is the key site of central nervous system (CNS) regulation of energy homeostasis and reproduction. These effects are mediated by interactions between neurotransmitters such as norepinephrine, dopamine, and neuropeptides, including neuropeptide Y (NPY), GnRH, corticotropin releasing factor (CRF), orexin, somatostatin, proopiomelanocortin (POMC), and peripheral hormones that act at the hypothalamus (e.g., leptin or insulin). The recently discovered *ob* gene product, leptin, a 16-kDa protein produced mainly by adipocytes, plays an important role in regulating feed intake, energy balance, and reproduction. It is well documented that leptin's central action is largely due to mediating hypothalamic NPY gene expression [1, 2]. Neuropeptide Y is also an important central regulator of food intake, energy expenditure, and reproductive function. The long form leptin receptor (Ob-Rl) is coexpressed in NPY neurons in the arcuate nucleus of the hypothalamus [3]. Leptin treatment inhibited NPY synthesis in the hypothalamus and, thus, reduced feed intake and increased energy expenditure [3]. However, hypothalamic NPY is not the only CNS target for leptin because NPY knockout mice responded to leptin treatment [4]. The leptin receptor is colocalized with several other neuropeptides within the hypothalamus such as CRF, POMC, and orexin [5, 6] that are involved in CNS regulation of feed intake, energy balance, and reproduction. In the present study, we investigated developmental changes in Ob-Rl, preproorexin, POMC, CRF, somatostatin, and GnRH gene expression in the hypothalamus and leptin mRNA expression in back fat in the pig.

MATERIALS AND METHODS

Animals

All animal experiments were approved by the Animal Care and Use Committee of the University of Georgia and the USDA Richard Russell Agricultural Research Center. Crossbred 106-day-old female fetus (n = 3) and 7-day-old (n = 3), 3.5-mo-old (n = 3), and 6-mo-old (n = 2) prepuberal gilts were used. These ages are associated with developmental changes in LH secretory patterns [7–10]. Pigs were satiated prior to euthanizing by an i.v. injection of sodium thiopental, except for the 6-mo-old animals that were fasted 12 h prior to exsanguination at a local abattoir. After exsanguination, tissues were collected from all pigs. The cranial vault was opened and the hypothalamus excised within 5–8 min after making the following cuts: rostral to the optic chiasm, rostral to the mammillary bodies, lateral to the hypothalamic sulci, and ventral to the anterior commissure. Dorsal subcutaneous adipose tissue was collected from a 106-day-old female fetus, and 7-day-old and 3.5-mo-old animals as previously described [11]. All tissue samples were frozen in liquid nitrogen and maintained at −80°C until RNA isolation.

RNA Isolation and Purification

Total RNA was extracted using Trizol reagent (Gibco, Grand Island, NY) according to the manufacture's procedure. Isolated RNA was treated with RQ1 RNase-free DNase (Promega, Madison, WI) to eliminate possible genomic DNA contamination. In brief, 10 μg of the RNA sample plus 5 μl 10× reaction buffer (400 mM Tris-HCl [pH 7.9], 100 mM NaCl, 60 mM $MgCl_2$, 100 mM $CaCl_2$), 5 units DNase, 1 μl RNasin (Promega, 40 U/μl), and RNase-free water was added to a final volume of 50 μl and incubated at 37°C for 30 min, then an equal volume of phenol/chlo-

[1]This research was supported by USDA funds and State and Hatch funds allocated to the Georgia Agricultural Experiment Station. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture or the University of Georgia and does not imply its approval to the exclusion of other products that may be suitable.
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Received: 17 March 2000.
First decision: 25 April 2000.
Accepted: 16 January 2001.

ISSN: 0006-3363. http://www.biolreprod.org

TABLE 1. Primer pairs for each selected gene, the annealing temperature, the length of PCR products, and the Genbank access number from which the primers were selected.

PCR product	Sense primer	Antisense primer	Temperature (°C)	Length (bp)	Access number
18s rRNA	5′-ACTGAGGCCATGATTAAG-3′	5′-GCTATCAATCTGTCAATCC-3′	52	400	gb|AF102857
Ob-Rl	5′-TCGGAAGATATCAGTGTTGA-3′	5′-TTGGGATGCTGATCTGATAA-3′	52	313	gb|AF36908
GnRH	5′-CTGGAGGAAAGAGAAATG-3′	5′-TTCAATCAGACTTTCCAGAG-3′	52	168	gb|L29342
POMC	5′-CGGTGAAGGTGTATCCCAAC-3′	5′-AGGTCATGAAGCCGCCGTAG-3′	54	267	gb|X01135
Somatostatin	5′-TTCTTGGCGGAGCTGCTGTC-3′	5′-GAGGAGAGGAATTGGAGTC-3′	56	245	gb|U36385
Preproorexin	5′-CGCTGCTGCTTCTGCTACTG-3′	5′-TCAGATCCCAGACCGTCCTC-3′	54	356	gb|AF075241
CRF	5′-GAATATTTCCTCCGCCTGGG-3′	5′-CTGTTGCTGTGAGCTTGCTG-3′	52	347	emb|Y15159
Leptin	5′-ACAGAGGGTCACCGGTTTGG-3′	5′-TAGAGGGAGGCTTCCAGGAC-3′	54	258	gb|AF026976

roform (Amresco, Solon, OH) was added. The mixture was vortexed and centrifuged at 12 000 × *g* for 15 min, the aqueous phase carefully transferred to a new Eppendorf microcentrifuge tube, and 1 ml 100% ethanol added and centrifuged at 12 000 × *g* for 10 min. The pellet was washed in 100% ethanol and centrifuged again. The pellet was air dried and resuspended in 20 μl RNase-free water and quantified by spectrophotometer (model DU640; Beckman, Fullerton, CA) at 260 nm and 280 nm. Quality of RNA was checked by electrophoresis using a 1% denatured agarose gel and stained with ethidium bromide.

Semiquantitative Reverse Transcription-Polymerase Chain Reaction

Two micrograms of total RNA was denatured with 500 ng oligo(dT) and 25 ng random primer at 70°C for 10 min and chilled on ice. Then, 4 μl 5× buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 15 mM $MgCl_2$), 2 μl 0.1 M dithiothreitol, 1 μl dNTP mixture (10 mM each), 1 μl Superscript II reverse transcriptase (Gibco), and 0.5 μl of RNasin (Promega) and RNase-free water were added to a final reaction volume of 20 μl. The tube was incubated at room temperature for 10 min and then transferred to 48°C for 50 min followed by additional 10 min at 70°C in a thermocycler (Gradient 40 robocycler; Stratagene, La Jolla, CA). After reverse transcription (RT), a 20-μl volume contained 2 μl of cDNA, 1 μl primer mixture (50 pmol each except 10 pmol each for 18s), 2 μl 10× buffer (500 mM KCl, 100 mM Tris-HCl [pH 9.0 at 25°C], 1% Triton X-100), 15 mM $MgCl_2$, 1 μl dNTP mixture (10 mM each), and 2.5 units *Taq* DNA polymerase (Promega) was used for the polymerase chain reaction (PCR). The PCR was performed at 1 cycle of 94°C for 3 min, specific annealing temperature for 1 min, and 72°C for 1 min, followed by 28 cycles at 94°C for 30 sec, specific annealing temperature for 1 min, and 72°C for 1 min and for 10 min in the last cycle. Specific annealing temperature and primers for each gene are presented in Table 1. Yield of 18s and ObR-l PCR products from 20–32 cycles was measured to determine the linear amplification range (Fig. 1). Negative controls were total RNA without reverse transcriptase. The PCR product for each specific gene was confirmed by its size and enzyme digestion. The PCR products were electrophoresed on a 2% agarose gel followed by ethidium bromide staining (0.4 μg/ml) and analyzed with an image analysis system (Flurochem; Alpha Innotech Corporation, San Leandro, CA) and related software (Flurochem version 1.02).

Data Analysis

Data from the image analysis were expressed as a ratio of Ob-Rl or neuropeptide mRNA relative to 18s rRNA. Expression data were subjected to square root transformation and one-way ANOVA according to the general linear model procedure of the Statistical Analysis System [12]. Differences between ages were determined by least-square contrasts. Linear regression was performed on the 18S rRNA and Ob-Rl mRNA amplification. A P value equal to or less than 0.05 was considered significant.

RESULTS

Data from image analysis are presented in Table 2, and an RT-PCR analysis of mRNA from a representative animal for each age is presented in Figure 2. Expression of Ob-Rl was greater ($P < 0.05$) at 3.5 mo and 6 mo of age than at the two earlier ages. Leptin mRNA expression in back fat was lower ($P < 0.01$) in 106-day-old fetuses than in 7-day-old and 3.5-mo-old gilts, whereas leptin mRNA was lower ($P < 0.01$) in 3.5-mo-old gilts than in 7-day-old gilts. Preproorexin mRNA expression was similar among 106-day-old fetuses and 7-day-old and 6-mo-old gilts but was greater ($P < 0.05$) in 3.5-mo-old gilts than in the other three age groups. The CRF mRNA expression was similar across ages. Somatostatin mRNA expression and GnRH mRNA expression was similar for 106-day-old fetuses, and 7-day-old and 6-mo-old gilts, whereas expression of mRNA for these neuropeptides was greater ($P < 0.02$) in 3.5-mo-old gilts compared to the other groups. The POMC mRNA expression was greater ($P < 0.01$) in 6-mo-old gilts compared to other groups.

DISCUSSION

The *ob* and *db* genes encode leptin [13] and its receptor [14], respectively. The action of leptin is mediated via Ob-

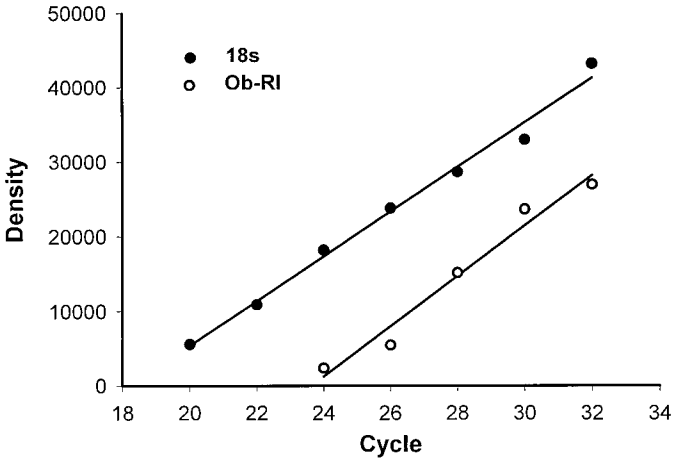

FIG. 1. Linear range of 18s rRNA and Ob-Rl mRNA PCR amplification. $R^2 = 0.993$ and 0.970, respectively.

TABLE 2. Hypothalamic expression of OB-Rl, preproorexin, CRF, somatostatin (SS), GnRH and POMC mRNA, and adipose tissue expression of leptin (LS mean ± SEM) for 106-day-old fetus (n = 3) and 7-day-old (n = 3), 3.5-mo-old (n = 3), and 6-mo-old (n = 2) gilts.[a]

Item	106-day-old fetus	7-day-old	3.5-mo-old	6-mo-old	*P* value
OB-Rl	0.15 ± 0.05[d]	0.19 ± 0.05[d]	0.44 ± 0.06[e]	0.38 ± 0.06[e]	<0.05
Preproorexin	0.70 ± 0.06[d]	0.59 ± 0.06[d]	0.96 ± 0.06[e]	0.75 ± 0.07[d]	<0.05
CRF	0.81 ± 0.17[d]	0.44 ± 0.21[d]	0.10 ± 0.17[d]	0.78 ± 0.21[d]	NS[b]
SS	0.52 ± 0.11[d]	0.62 ± 0.11[d]	1.32 ± 0.13[e]	0.36 ± 0.13[d]	<0.02
GnRH	0.21 ± 0.03[d]	0.19 ± 0.03[d]	0.74 ± 0.04[e]	0.38 ± 0.04[d]	<0.02
POMC	0.31 ± 0.05[d]	0.30 ± 0.05[d]	0.26 ± 0.05[d]	0.73 ± 0.06[e]	<0.01
Adipose tissue leptin	0.18 ± 0.03[d]	0.71 ± 0.03[e]	0.52 ± 0.03[f]	ND[c]	<0.01

[a] Expressed as a ratio of Ob-Rl, neuropeptide or leptin mRNA relative to 18s rRNA.
[b] NS, Nonsignificant.
[c] ND, Not determined.
[d,e,f] Means in a row with different superscripts differ; *P*, level of significance.

Rl in the hypothalamus [15]. Leptin mRNA expression in adipose tissue was detectable in 106-day-old fetuses as was Ob-Rl expression in hypothalamic tissue. We previously observed Ob-Rl expression in adipose tissue from a 50-day-old fetus [16]. Taken together these data suggest that leptin may play a role during fetal development. The Ob-Rl mRNA expression in the hypothalamus increased by 3.5 mo of age and remained elevated at 6 mo of age relative to 106-day-old fetuses and 7-day-old gilts, demonstrating an age-dependent increase in Ob-Rl expression. This occurred at a time when serum leptin concentrations increased in the developing pig [17]. In the pig a constant ad libitum intake of feed was established between 4 and 6 mo of age, in advance of attainment of mature size [18]. Thus, changes in serum leptin concentrations, coupled with Ob-Rl expression in the hypothalamus, may, in part, explain the age-related change in feed intake.

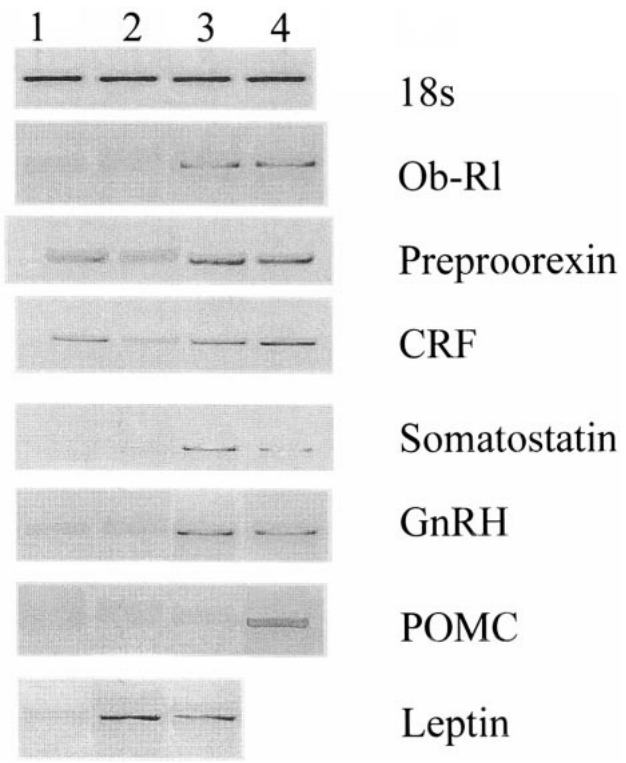

FIG. 2. The RT-PCR analysis of 18s rRNA, Ob-Rl, GnRH, POMC, somatostatin, preproorexin, CRF mRNA in hypothalamic tissue and leptin mRNA in adipose tissue from a representative animal for each age. Lanes 1, 2, 3, and 4 represent 106-day-old fetus, 7-day-old, 3.5-mo-old, and 6-mo-old female pig, respectively.

The early growth pattern of the gilt is linear. Growth sharply increased from the postnatal to the peripubertal period [19]. However, serum GH concentrations and pituitary response to GH releasing factor declined with age in the pig [20]. Greater hypothalamic somatostatin mRNA expression at 3.5 mo of age compared to the other ages supports the idea of an age-related decline in GH secretion related to increased somatostatin secretion. Furthermore, Drisko et al. [21] reported a temporal relationship between hypophyseal-portal blood concentration of somatostatin and generation of serum GH pulses in the pig.

In general, in the gilt, mean serum LH concentration and serum LH pulse frequency increased from 15 days of age to maximum levels between 110 and 125 days of ages, then decreased until 150 days of age and remained suppressed (juvenile nadir) until the peripuberal period [7–10]. There is little information regarding mechanisms within the brain that brings the various components of the reproductive axis together in a proper temporal relationship to initiate puberty [22–25]. Several studies have characterized LH secretion in the pig during pubertal development. In the present study, GnRH mRNA expression increased markedly by 3.5 mo of age and declined by 6 mo of age. This expression pattern is consistent with the LH secretory pattern observed during development; particularly around the time of the juvenile nadir. A recent report by Morash et al. [26] demonstrated that leptin mRNA is selectively expressed in specific areas in the brain and pituitary in the rat. Leptin gene was expressed in the hypothalamus, and expression was regulated by nutrient availability. The authors suggested that central leptin mRNA expression may play a role in appetite control. It is possible that central leptin may be involved in maturation of the GnRH/LH secretory axis.

In the present study, CRF expression did not change over the ages studied. This is consistent with an early report by Emanuel et al. [27] indicating that CRF mRNA expression in the rat hypothalamus did not change during development. However, there could be posttranscriptional regulation of the CRF gene product in rats [27] as well as in pigs.

Other substances may be intermediates in the signal transduction pathway between leptin and GnRH secretion. Neurons containing POMC and its products, i.e., ACTH, β-endorphin, and melanocyte stimulating hormones (MSHs), are located in areas within the hypothalamus that are involved in GnRH secretion and feed intake regulation in the pig [28, 29]. Direct synaptic contacts between POMC- and GnRH-containing neurons were found in the arcuate nucleus of the hypothalamus, a region high in Ob-Rl mRNA [30–32]. Leptin treatment reduced feed intake and increased POMC mRNA in *ob/ob* mice [33] and fasting

decreased POMC mRNA expression in rodents [34, 35]. In the pig, morphine suppressed LH secretion after intracerebroventricular (ICV) administration [36] and inhibited GnRH release from hypothalamic tissue in vitro [37]. Thus, the potential exists for leptin, through activation of the POMC gene, to influence LH secretion. Furthermore, recent studies demonstrated that MSH acts to inhibit feed intake via the melanocortin-4-receptor [38]. In the present study, POMC mRNA expression increased by 6 mo of age, a time when GnRH expression was suppressed. This increased expression in POMC may contribute to changes in feed intake and the juvenile nadir in LH secretion described above.

Orexins (Orexin-A and -B) are neuropeptides synthesized in the hypothalamus and derived from preproorexin. Preproorexin mRNA expression is located in the dorsal and lateral hypothalamic areas of the rat brain [39]. Orexin neurons send projections to multiple targets including the arcuate nucleus and the preoptic area [39, 40]. Horvathe et al. [41] colocalized Ob-R on orexin-containing neurons. In addition, orexin neurons innervate POMC and NPY neurons, suggesting that orexin not only modulates feed intake but also regulates neuroendocrine function. Orexin treatment stimulated food intake while fasting increased orexin expression in the rat [42]. Moreover, ICV administration of orexin stimulated LH secretion in steroid-treated ovariectomized rats [43]. Preproorexin gene expression was high at 3.5 mo of age compared to the other ages in the current study. This occurred at a time when LH secretion is relatively high in the pig [8].

In summary, although these findings are correlative in nature, the data suggest that Ob-Rl and a number of hypothalamic and peripheral peptides may be associated with development of the neuroendocrine axis and may serve as messengers that link mechanisms that regulate reproduction and energy balance. However, future studies are needed to demonstrate that the mRNA is translated into protein product before definitive conclusions can be made.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Rick Meinersmann for using his facilities and Mr. Benny Barrett for his technical help.

REFERENCES

1. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996; 98:1101–1106.
2. Hakansson ML, Hulting AL, Meister B. Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus—relationship with NPY neurones. Neuroreport 1996; 7:3087–3092.
3. Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Morgan PJ, Trayhurn P. Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. J Neuroendocrinol 1996; 8:733–735.
4. Erickson JC, Clegg KE, Palmiter RD. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 1996; 381:415–421.
5. Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 1998; 18:559–572.
6. Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 1999; 23:775–786.
7. Lutz JB, Rampacek GB, Kraeling RR, Pinkert CA. Serum luteinizing hormone and estrogen profiles before puberty in the gilt. J Anim Sci 1984; 58:686–691.
8. Pelletier J, Carrez-Camous S, Thiery JC. Basic neuroendocrine events before puberty in cattle, sheep and pigs. J Reprod Fertil Suppl 1981; 30:91–102.
9. Camous S, Prunier A, Pelletier J. Plasma prolactin, LH, FSH and estrogen excretion patterns in gilts during sexual development. J Anim Sci 1985; 60:1308–1317.
10. Diekman MA, Trout WE, Anderson LL. Serum profiles of LH, FSH and prolactin from 10 weeks of age until puberty in gilts. J Anim Sci 1983; 56:139–145.
11. Hausman GH, Wright JT, Richardson RL. The influence of extracellular matrix on preadiopcyte development in serum-free cultures of stromal-vascular cells. J Anim Sci 1996; 74:2117–2128.
12. SAS. SAS/STAT Guide for Personal Computers. Cary, NC: Statistical Analysis System Institute Inc.; 1987.
13. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372:425–432.
14. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muri C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monore CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. Cell 1995; 83:1263–1271.
15. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 395:763–770.
16. Lin J, Barb CR, Matteri RL, Kraeling RR, Chen X, Rampacek GB, Meinersmann RJ. Leptin receptor mRNA expression in the brain, pituitary and other tissues in the pig. J Anim Sci 1999; 77:217 (abstract).
17. Qian H, Barb CR, Compton MM, Hausman GJ, Azain MJ, Kraeling RR, Baile CA. Leptin mRNA expression and serum leptin concentrations as influenced by age, weight, and estradiol in pigs. Domest Anim Endocrinol 1999; 16:135–143.
18. Reeds PJ, Burrin DG, Davis TA, Fiototto MA, Mersmann HJ, Pond WG. Growth regulation with particular reference to the pig. In: Hollis GR (ed.), Growth of the Pig. Wallingford, UK: CAB International; 1993: 10–11.
19. Robison OW. Growth patterns in swine. J Anim Sci 1976; 42:1024–1035.
20. Dubreuil P, Pelletier G, Petitclerc D, Lapierre H, Couture Y, Brazeau P, Gaudreau P, Morisset J. Influence of age and sex on basal secretion of growth hormone (GH) and on GH-induced release by porcine GH-releasing factor pGRF(1–29NH2) in growing pigs. Domest Anim Endocrinol 1987; 4:299–307.
21. Drisko JE, Faidley TD, Chang CH, Zhang D, Nicolich S, Hora DFJ, McNamara L, Rickes E, Abribat T, Smith RG, Hickey GJ. Hypophyseal-portal concentrations of growth hormone-releasing factor and somatostatin in conscious pigs: relationship to production of spontaneous growth hormone pulses. Proc Soc Exp Biol Med 1998; 217:188–196.
22. Clapper JA, Green ML, Diekman MA. Serum concentrations of immunoactive and bioactive luteinizing hormone (LH) in gilts. II. Biopotency of LH increases at puberty but remains constant throughout the estrous cycle. Biol Reprod 1993; 49:750–756.
23. Clapper JA, Green ML, Diekman MA. Serum concentrations of immunoactive and bioactive luteinizing hormone (LH) in gilts. I. Biopotency of LH increases during prepubertal development. Biol Reprod 1993; 49:757–763.
24. Prunier A, Chopineau M, Mounier AM, Mormede P. Patterns of plasma LH, FSH, oestradiol and corticosteroids from birth to the first oestrous cycle in Meishan gilts. J Reprod Fertil 1993; 98:313–319.
25. Esbenshade KL, Paterson AM, Cantley TC, Day BN. Changes in plasma hormone concentrations associated with the onset of puberty in the gilt. J Anim Sci 1982; 54:320–324.
26. Morash B, Li A, Murphy PR, Wilkinson M, Ur E. Leptin gene expression in the brain and pituitary gland. Endocrinology 1999; 140: 5995–5998.
27. Emanuel RL, Thull DL, Girard DM, Majzoub JA. Developmental expression of corticotropin releasing hormone messenger RNA and peptide in rat hypothalamus. Peptides 1989; 10:1165–1169.
28. Kineman RD, Kraeling RR, Crim JW, Leshin LS, Barb CR, Rampacek GB. Localization of proopiomelanocortin (POMC) immunoreactive neurons in the forebrain of the pig. Biol Reprod 1989; 40:1119–1126.
29. Kineman RD, Leshin LS, Crim JW, Rampacek GB, Kraeling RR. Localization of luteinizing hormone-releasing hormone in the forebrain of the pig. Biol Reprod 1988; 39:665–672.
30. Leranth C, MacLusky NJ, Shanabrough M, Naftolin F. Immunohistochemical evidence for synaptic connections between pro-opiomelanocortin-immunoreactive axons and LH-RH neurons in the preoptic area of the rat. Brain Res 1988; 449:167–176.
31. Thind KK, Goldsmith PC. Infundibular gonadotropin-releasing hor-

mone neurons are inhibited by direct opioid and autoregulatory synapses in juvenile monkeys. Neuroendocrinology 1988; 47:203–216.
32. Chen WP, Witkin JW, Silverman AJ. Beta-endorphin and gonadotropin-releasing hormone synaptic input to gonadotropin-releasing hormone neurosecretory cells in the male rat. J Comp Neurol 1989; 286: 85–95.
33. Thornton JE, Cheung CC, Clifton DK, Steiner RA. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 1997; 138:5063–5066.
34. Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 1997; 46:2119–2123.
35. Mizuno TM, Kleopoulos SP, Bergen HT, Roberts JL, Priest CA, Mobbs CV. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and in ob/ob and db/db mice, but is stimulated by leptin. Diabetes 1998; 47:294–297.
36. Barb CR, Kineman RD, Kesner JS, Rampacek GB, Kraeling RR. Luteinizing hormone secretion following intracerebroventricular administration of morphine in the prepuberal gilt. Life Sci 1989; 45:691–696.
37. Barb CR, Chang WJ, Leshin LS, Rampacek GB, Kraeling RR. Opioid modulation of gonadotropin releasing hormone release from the hypothalamic preoptic area in the pig. Domest Anim Endocrinol 1994; 11:375–382.
38. Flier JS, Maratos-Flier E. Obesity and the hypothalamus: novel peptides for new pathways. Cell 1998; 92:437–440.
39. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573–585.
40. De Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998; 95:322–327.
41. Horvath TL, Diano S, van den Pol AN. Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. J Neurosci 1999; 19:1072–1087.
42. Cai XJ, Widdowson PS, Harrold J, Wilson S, Buckingham RE, Arch JR, Tadayyon M, Clapham JC, Wilding J, Williams G. Hypothalamic orexin expression: modulation by blood glucose and feeding. Diabetes 1999; 48:2132–2137.
43. Pu S, Jain MR, Kalra PS, Kalra SP. Orexins, a novel family of hypothalamic neuropeptides, modulate pituitary luteinizing hormone secretion in an ovarian steroid-dependent manner. Regul Pept 1998; 78: 133–136.